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ROLE OF TELOMERES AND TELOMERASE IN AGING

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ABSTRACT

Telomeres are sequences of DNA chains of chemical code. Telomeres are made of repeating sequences of TTAGGG on one strand paired with AATCCC on the other strand. Cells normally can divide only about 50 to 70 times, with telomeres getting progressively shorter until the cells become senescent or die. Telomere is the biologic time clock at the end of your chromosomes. Telomerase is a ribonucleoprotein that is an enzyme that adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the 3' end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes. Telomerase has many diagnostic, therapeutic and prognostic potential applications for the future. If the telomerase is reactivated in cells that have gone into senescence, the cells could continue to divide and bypass cellular aging. Telomerase could also be used to slow the rate of telomere loss and as a result extend cell life span.

KEYWORDS: Telomeres, telomerase, ribonucleoprotein, chromosomes.

INTRODUCTION TO TELOMERES

Like the rest of a chromosome, including its genes, telomeres are sequences of DNA — chains of chemical code. Like all DNA, they are made of four nucleic acid bases: G for guanine, A for adenine, T for thymine, and C for cytosine. Telomeres are made of repeating sequences of TTAGGG on one strand paired with AATCCC on the other strand. Thus, one section of telomere is a "repeat" made of six "base pairs." In white blood cells, the length of telomeres ranges from 8,000 base pairs in newborns to 3,000 base pairs in adults and as low as 1,500 in elderly people.[1] (An entire chromosome has about 150 million base pairs.) Each time it divides, an average cell loses 30 to 200 base pairs from the ends of its telomeres. Cells normally can divide only about 50 to 70 times, with telomeres getting progressively shorter

until the cells become senescent or die. Telomeres do not shorten in tissues where cells do not continually divide, such as heart muscle.[2]

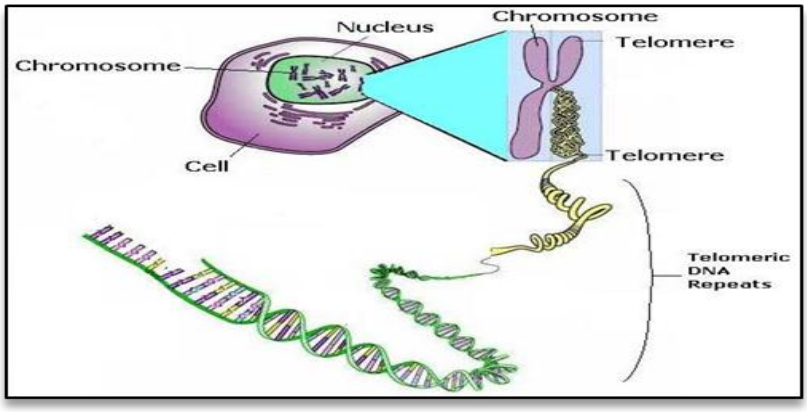
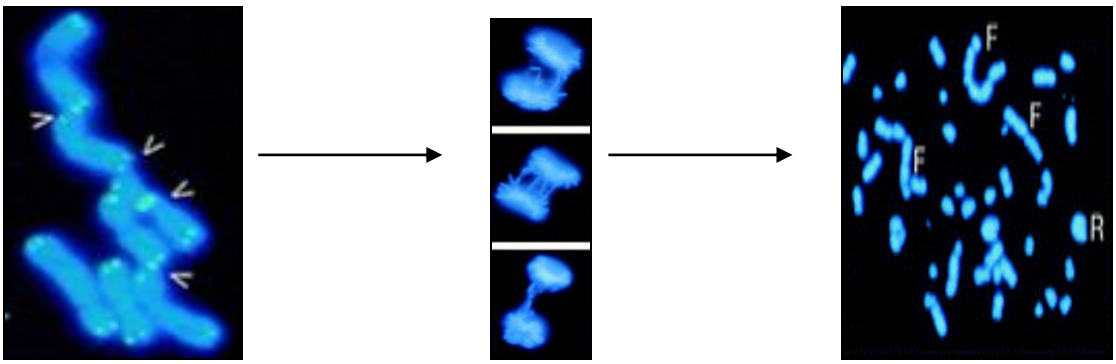


Fig: 1. Position of telomere in the chromosome

WHY DO CHROMOSOMES HAVE TELOMERES?

Telomere: The biologic time clock at the end of your chromosomes. Every time a cell divides it loses a piece of this "time clock" and the life of the cell shortens thus shortening your life! Without telomeres, the main part of the chromosome — the part with genes essential for life — would get shorter each time a cell divides. So telomeres allow cells to divide without losing genes. Cell division is necessary for growing new skin, blood, bone, and other cells. Without telomeres, chromosome ends could fuse together and corrupt the cell's genetic blueprint, possibly causing malfunction, cancer, or cell death. Because broken DNA is dangerous, a cell has the ability to sense and repair chromosome damage.[3] Without telomeres, the ends of chromosomes would look like broken DNA, and the cell would try to fix something that wasn't broken, stop dividing and eventually die.



Telomeric fusion 'Bridge' chromosomes Chromosome breakage Fusion of broken chromosome

Fig: 2. Fusion-bridge-breakage cycles

HOW BIG A ROLE DO TELOMERES PLAY IN AGING?

Leonard Hayflick first described cellular aging in detail in 1961. He explained that most normal human cells are mortal because they can divide only a finite number of times.In culture, cells divide from 20 to 100 times before they stop. The limit of cell reproduction is often referred to as the "Hayflick Limit."Since Hayflick's discoveries many theories have been formed to explain the mechanism behind cell aging.

The connection between telomeres and aging first emerged in 1986 from observations made by Howard Cooke. Cooke noticed that the length of telomeres in reproductive cells were longer than telomeres in somatic cells. Since Cooke understood that telomeres shortened each time that a cell divided, he concluded that somatic cells must not make telomerase.

Cooke's discovery coincides with one of the theories used to describe aging, the telomere hypothesis. This theory proposes that as mortal cells divide, they eventually lose parts of their telomeres until they ultimately die. Only cells that can continue to reactivate their telomerase will continue to live. Cells that cannot reactivate their telomerase will stop dividing, or senesce. In the following model of the telomere hypothesis the terminal restrictive fragment (TRF) is plotted against the age of replication.

An estimate of telomere length is measured by digesting cellular DNA with restriction enzymes having 4-base recognition sites, so that most of the DNA is reduced to short fragments. Because telomere repeats lack restriction sites, they remain as relatively long terminal restrictive fragments.

In 1997, Thomas Cech and colleagues at Geron Corporation isolated the human gene for a catalytic protein called telomerase reverse transcriptase (hTRT). This gene is only found in immortal cells. The function of the hTRT is to add the repeating sequences to the chromosomes, lengthening the telomeres. Cawthon's study found that when people are divided into two groups based on telomere length, the half with longer telomeres lives an average of five years longer than those with shorter telomeres. This study suggests that lifespan could be increased five years by increasing the length of telomeres in people with shorter ones. People with longer telomeres still experience telomere shortening as they age. How many years might be added to our lifespan by completely stopping telomere shortening? Cawthon believes 10 years and perhaps 30 years.[4]

After age 60, the risk of death doubles every 8 years. So a 68-year-old has twice the chance of dying within a year compared with a 60-year-old. Cawthon's study found that differences in telomere length accounted for only 4% of that difference. And while intuition tells us older people have a higher risk of death, only 6% is due purely to chronological age. When telomere length, chronological age, and gender are combined (women live longer than men), those factors account for 37% of the variation in the risk of dying over age 60. So what causes the other 63%?

CAUSES OF AGING

A major cause of aging is "oxidative stress." It is the damage to DNA, proteins, and lipids (fats) caused by oxidants, which are highly reactive substances containing oxygen. These oxidants are produced normally when we breathe, and also result from inflammation, infection, and consumption of alcohol and cigarettes. In one study, scientists exposed worms to two substances that neutralize oxidants, and the worms' lifespan increased an average 44%. Another factor in aging is "glycation." It happens when glucose, the main sugar we use as energy, binds to some of our DNA, proteins, and lipids, leaving them unable to do their jobs. The problem becomes worse as we get older, causing body tissues to malfunction, resulting in disease and death. Glycation may explain why studies in laboratory animals indicate that restricting calorie intake extends lifespan. Most likely oxidative stress, glycation, telomere shortening, and chronological age — along with various genes — all work together to cause aging.[5]

TELOMERES AND OTHER DISEASES

People with a disease named dyskeratosis congenita have telomeres that get short much more quickly than normal. These people endure premature aging and death. They face a higher risk of life-threatening infections, leukemia and other blood cancers, intestinal disorders, cirrhosis of the liver, and pulmonary fibrosis, a deadly stiffening of lung tissue. They also are more likely to endure gray hair, balding, poor wound healing, spots on the skin, intestinal disorders, softening of the bones, and learning disabilities. The implication is that telomeres may play a role in all those conditions, because they all involve tissues in which cells divide often. There also is some evidence linking shortened telomeres to Alzheimer disease, hardening of the arteries, high blood pressure, and type 2 diabetes.[6]

THE PROCESS OF DNA REPLICATION

DNA replication is the process of producing two identical replicas from one original DNA molecule. This biological process occurs in all living organisms and is the basis for biological inheritance. DNA is made up of two strands and each strand of the original DNA molecule serves as template for the production of the complementary strand, a process referred to as semiconservative replication. Cellular proofreading and error-checking mechanisms ensure near perfect fidelity for DNA replication.

In a cell, DNA replication begins at specific locations, or origins of replication, in the genome. Unwinding of DNA at the origin and synthesis of new strands results in replication forks growing bidirectionally from the origin. A number of proteins are associated with the replication fork which helps in terms of the initiation and continuation of DNA synthesis. Most prominently, DNA polymerase synthesizes the new DNA by adding complementary nucleotides to the template strand. DNA replication can also be performed *in vitro* (artificially, outside a cell). DNA polymerases isolated from cells and artificial DNA primers can be used to initiate DNA synthesis at known sequences in a template DNA molecule. The polymerase chain reaction (PCR), a common laboratory technique, cyclically applies such artificial synthesis to amplify a specific target DNA fragment from a pool of DNA.

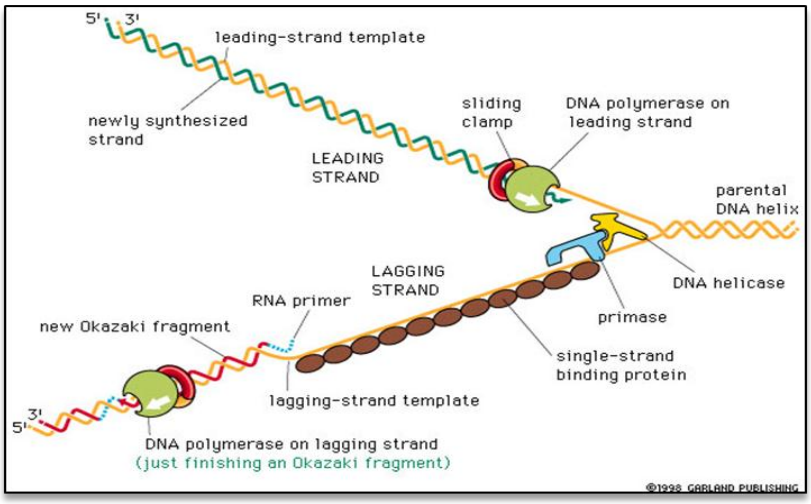


Fig: 2. Process of DNA replication

TELOMERE LENGTH AND AGING

At birth, the average human telomere length is 10,000 base pairs. At 20 years, the average telomere length is 8,000. By age 50, around 7,000 remain. And so it goes, losing 50-100 base pairs a year until only 4000 remain at age 100.

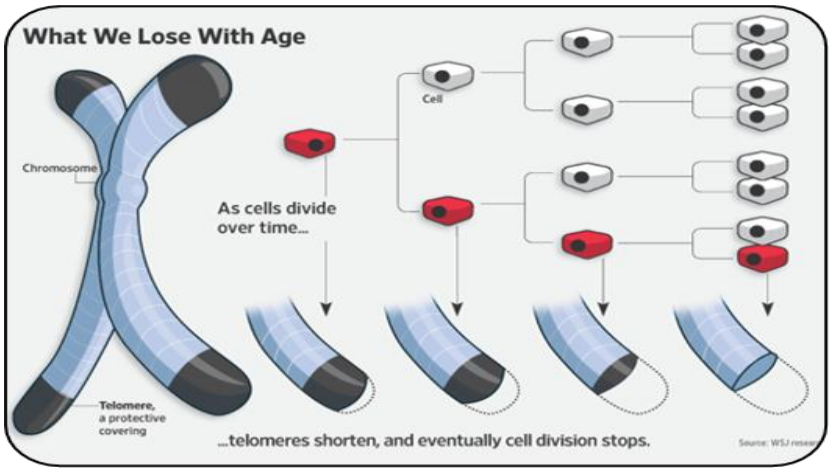


Fig: 4. Telomere length and aging

TELOMERASE

Telomerase is a ribonucleoprotein that is an enzyme that adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the 3' end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes. This region of repeated nucleotide called telomeres contains noncoding DNA and hinders the loss of important DNA from chromosome ends. As a result, every time the chromosome is copied, only 100–200 nucleotides are lost, which causes no damage to the organism's DNA. Telomerase is a reverse transcriptase that carries its own RNA molecule, which is used as a template when it elongates telomeres, which are shortened after each replication cycle.

Telomerase, also called telomere terminal transferase, is an enzyme made of protein and *RNA* subunits that elongates chromosomes by adding TTAGGG sequences to the end of existing chromosomes. Telomerase is found in fetal tissues, adult *germ cells*, and also tumor cells. Telomerase activity is regulated during development and has a very low, almost undetectable activity in *somatic* (body) cells. Because these somatic cells do not regularly use telomerase, they age. The result of aging cells is an aging body. If telomerase is activated in a cell, the cell will continue to grow and divide.

Cellular aging, or *senescence*, is the process by which a cell becomes old and dies. It is due to the shortening of chromosomal telomeres to the point that the chromosome reaches a critical length. Cellular aging is analogous to a wind up clock. If the clock stays wound, a cell becomes immortal and constantly produces new cells. If the clock winds down, the cell stops producing new cells and dies. Our cells are constantly aging. Being able to make the body's

cells live forever certainly creates some exciting possibilities. Telomerase research could therefore yield important discoveries related to the aging process.

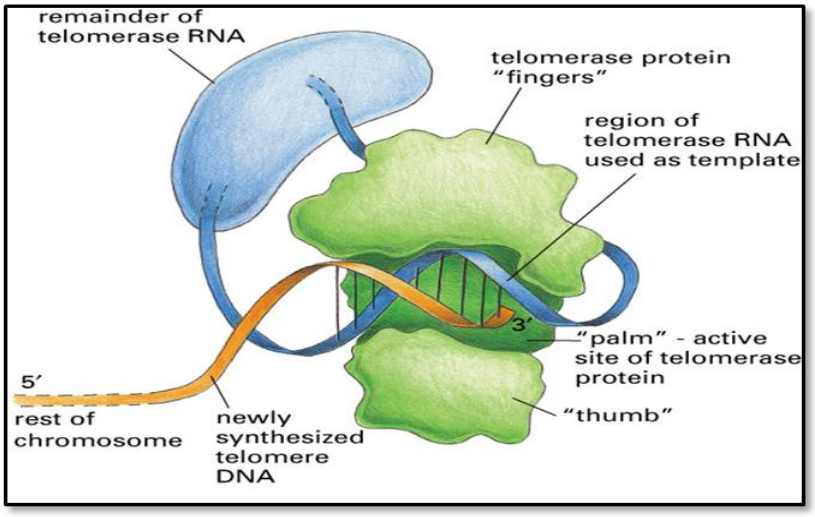


Fig: 5. Structure of Telomerase

hTRT – EXPERIMENT

The group introduced the hTRT into mortal cells. The outcome of the experiment was the production of active telomerase and the extended life of the originally mortal cells. These findings show that by reactivating telomerase activity in cells, cellular aging can be bypassed. Experiments have been performed on somatic cells from newborns and seventy-year olds. The cells derived from the newborns were found to divide 80 to 90 times in culture while the cells derived from the seventy-year olds are likely to divide only 20 to 30 times. When human cells that are normally capable of dividing, stop reproducing they look different and function less efficiently, and eventually they die.

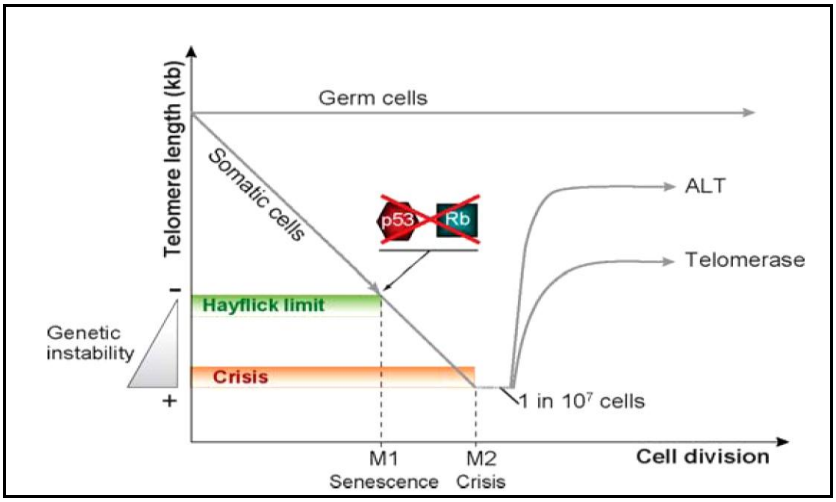


Fig: 6. Experimental result on Telomerase

ROLE OF TELOMERASE

Telomerase places one strand of DNA on the RNA, positioning itself so that the template lies adjacent to the tip of the chromosome. Then, the enzyme adds one DNA nucleotide at a time until a full telomeric subunit is formed. When the subunit is complete, telomerase can attach another by sliding to the new end of the chromosome and repeating the synthetic process.

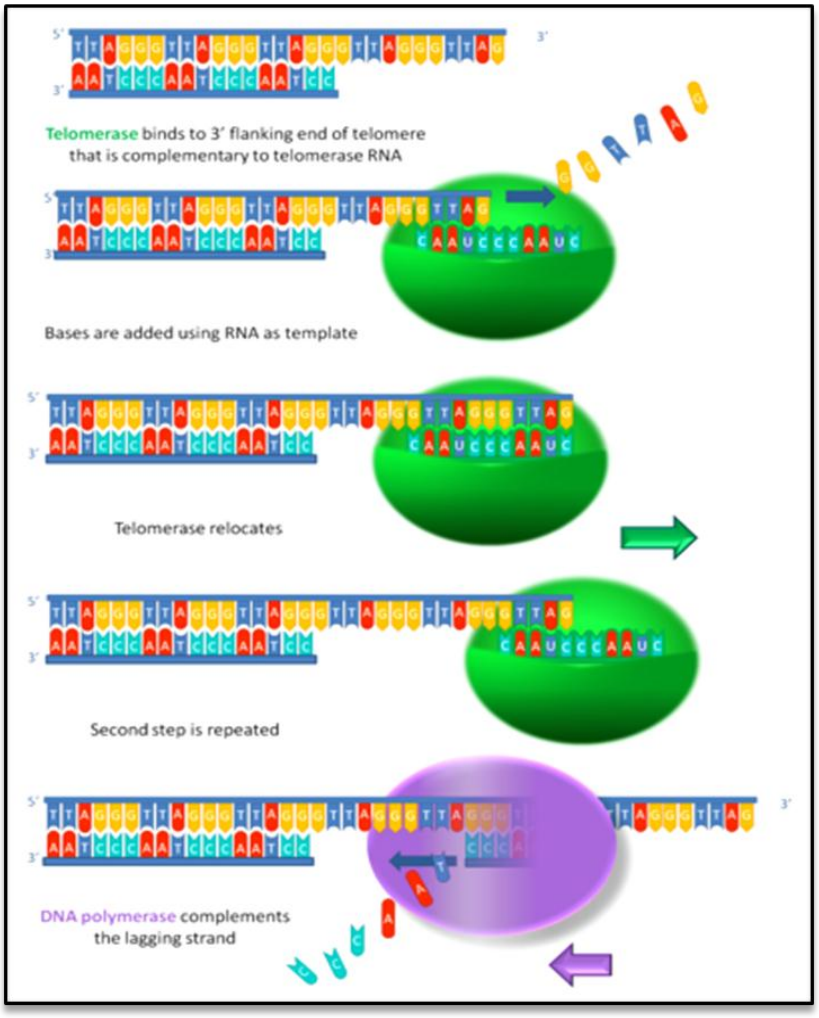


Fig: 6. Function of Telomerase

MEASURE TELOMERE LENTH BY USING qPCR

Cells in all organisms regulate gene expression by turnover of gene transcripts (messenger RNA, abbreviated to mRNA): The amount of an expressed gene in a cell can be measured by the number of copies of an mRNA transcript of that gene present in a sample. In order to robustly detect and quantify gene expression from small amounts of RNA, amplification of the gene transcript is necessary. The polymerase chain reaction (PCR) is a common method

for amplifying DNA; for mRNA-based PCR the RNA sample is first reverse-transcribed to cDNA with reverse transcriptase.[6]

In order to amplify small amounts of DNA the same methodology is used as in conventional PCR using a DNA template, at least one pair of specific primers, deoxyribonucleotides, a suitable buffer solution and a thermo-stable DNA polymerase. A substance marked with a fluorophore is added to this mixture in a thermal cycler that contains sensors for measuring the fluorescence of the flurophore after it has been excited at the required wavelength allowing the generation rate to be measured for one or more specific products. This allows the rate of generation of the amplified product to be measured at each PCR cycle. The data thus generated can be analysed by computer software to calculate *relative gene expression* (or *mRNA copy number*) in several samples. Quantitative PCR can also be applied to the detection and quantification of DNA in samples to determine the presence and abundance of a particular DNA sequence in these samples. This measurement is made after each amplification cycle, and this is the reason why this method is called real time PCR (that is, immediate or simultaneous PCR). In the case of RNA quantitation, the template is complementary DNA (cDNA), which is obtained by reverse transcription of ribonucleic acid (RNA). In this instance the technique used is quantitative RT-PCR or Q-RT-PCR.[7]

The most commonly used normalizing genes are those that code for the following proteins: tubulin, glyceraldehyde-3-phosphate dehydrogenase, albumin, cyclophilin, and ribosomal RNAs.

BASIC PRINCIPLES

Quantitative PCR is carried out in a thermal cycler with the capacity to illuminate each sample with a beam of light of a specified wavelength and detect the fluorescence emitted by the excited fluorophore. The thermal cycler is also able to rapidly heat and chill samples, thereby taking advantage of the physicochemical properties of the nucleic acids and DNA polymerase.[8]

The PCR process generally consists of a series of temperature changes that are repeated 25 – 40 times, these cycles normally consist of three stages: the first, at around 95 °C, allows the separation of the nucleic acid's double chain; the second, at a temperature of around 50-60 °C, allows the binding of the primers with the DNA template; the third at between 68 - 72 °C, facilitates the polymerization carried out by the DNA polymerase. Due to the small

size of the fragments the last step is usually omitted in this type of PCR as the enzyme is able to increase their number during the change between the alignment stage and the denaturing stage. In addition, some thermal cyclers add another short temperature phase lasting only a few seconds to each cycle, with a temperature of, for example, 80 °C, in order to reduce the noise caused by the presence of primer dimers when a non-specific dye is used. The temperatures and the timings used for each cycle depend on a wide variety of parameters, such as: the enzyme used to synthesize the DNA, the concentration of divalent ions and deoxyribonucleotides (dNTPs) in the reaction and the bonding temperature of the primers.[8]

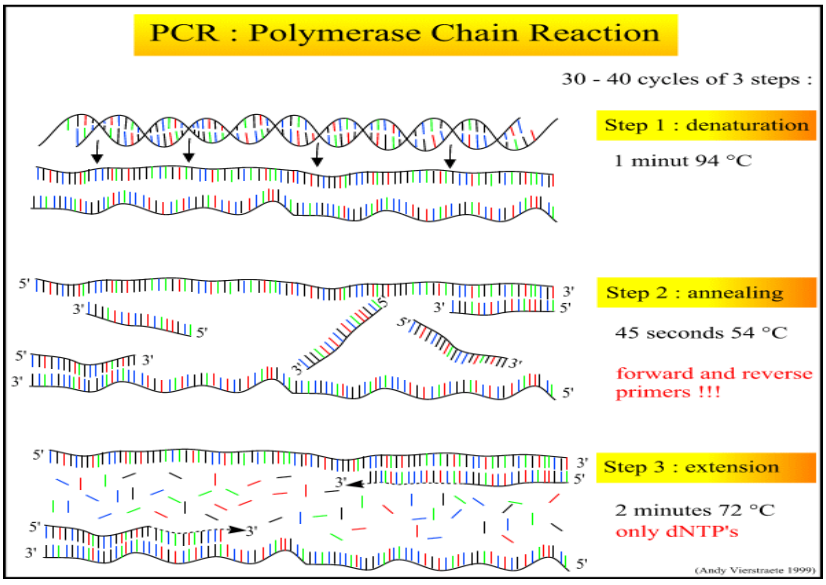


Fig: 5. Polymerase Chain Reaction

BENEFITS OF TELOMERE TEST

The primary benefit of a telomere test may be the information it provides to an individual regarding their biological age and susceptibility of aging and degenerative diseases.

Markers for biological age.

This information may enable a person to make adjustments to his/her lifestyle that can lead to better health and a longer life.

Telomere shortening is related to aging, degenerative disease, cardiovascular disease, stress and causes impair immune function that might also increase cancer susceptibility.[9]

RECENT TRENDS

There are various companies that are involved in manufacturing products related to human telomerase.

The Geron Company received a patent relating to the RNA component of human telomerase in 1997. They believe that by combining the RNA component with the hTRT gene product they could immortalize cells without turning them cancerous. If this technique works, not only would the possibility to rejuvenate human cells to treat age-related diseases be more feasible, but also other technologies that are currently limited by the mortality of normal human cells.

TA-65 is produced at very low levels in the astragalus plant, but the company purifies and concentrates the substance, which is thought to "turn on" the enzyme telomerase (hTERT) that acts to maintain or lengthen telomeres. hTERT is usually "off" in adult cells, except in immune, egg and sperm cells, and in malignant cancer-forming cells. The TA-65 pill requires no approval from the U.S. Food and Drug Administration because it is marketed as a supplement and not a drug.[10]

FUTURE PROSPECTS

One of the potential applications of telomerase is to postpone cellular aging by delaying critical telomere loss therapeutically, through reactivation of telomerase. Telomerase has many diagnostic, therapeutic and prognostic potential applications for the future. If the telomerase is reactivated in cells that have gone into senescence, the cells could continue to divide and bypass cellular aging. Telomerase could also be used to slow the rate of telomere loss and as a result extend cell life span. This could reduce the impact of senescence on the human body as well as on diseases that occur as a result of cell senescence.

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